FYI-0803-01424

American Chemistry Council
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August 8, 2003

VIA E-MAIL AND REGULAR MAIL

Richard Hefter, Chief
High Production Volume Chemicals Branch
c/o Document Processing Center (7407M)
EPA East-Room 6428 Attn: FYI
Office of Pollution, Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Contain NO CBI

Re: FYI-0102-01424 Regarding Results of a 2001 90-Day
Rat Oral Toxicity Study of Hexabromocyclododecane (HBCD)

Dear Mr. Hefter:

This reply to your July 10, 2003 correspondence is being submitted by the American Chemistry Council's (ACC) Brominated Flame Retardants Industry Panel (BFRIP). In your correspondence you requested BFRIP's response to comments provided by EPA concerning the conclusions reached in a 90-day oral gavage toxicity study of hexabromocyclododecane (HBCD) in rats that was submitted voluntarily by BFRIP. Your comments concerned the conclusions reached in the study regarding the NOAEL of 1000 mg/kg/day. BFRIP's response to the EPA's comments provided in your letter is provided in Attachment 1.

You also asked that we advise the Agency of any revised conclusions concerning this study, including any potential reportability pursuant to TSCA § 8(e). For the reasons discussed in Attachment 2, the conclusions regarding this study have not changed since our original submittal.

If you have any additional questions concerning the HBCD study, please contact me at 703/741-5639 or by e-mail at [wendy_sherman@americanchemistry.com].

Sincerely,

Wendy Sherman
Panel Manager, BFRIP

Attachments (2)

cc: Dr. Jenny Tao
BFRIP TTG Members

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ATTACHMENT 1

Point 1: Statistically significant ($p<0.05$ or $p<0.01$) decreased T4 level accompanied by histopathological changes in the thyroid glands in all male dose groups and in the 300 and 1000 mg/kg/day female groups.

Please see pages 56-57 of the final report.

The reduction in serum T4 levels detected in this study, while measurable, did not result in adverse effects in the treated animals. The rats grew and behaved, and were observed to be normal for this age and strain of rats. Thyroid hormone levels are not typically measured in subchronic studies, and in the absence of clinical changes indicative of thyroid disease, these rats would not have been diagnosed as having abnormal thyroid function. Further, the final report on the 90-day study concludes that the decrease in serum T4 at week 13 was related to its enhanced elimination due to induction of hepatic UDPG. The increase in liver weight, the lack of adverse histologic effects in the liver and the apparent normal functioning of the liver are all consistent with enzyme induction (Amacher et al. 1998). Hepatic enzyme induction is an adaptive, and not an adverse, effect (Williams and Iatropoulos 2002). The rat is particularly sensitive to hepatic enzyme induction; whereas the human is not, and enhanced T4 elimination due to enzyme induction is not applicable to human risk characterization (Capen 1997).

The reversible histologic changes in the thyroid consisted of a slight increase in the incidence of minimal follicular cell hypertrophy in the high dose males and minimal or mild hypertrophy in the high dose females. The final report concluded that it was not readily apparent that these minimal changes were an effect of treatment. The final report further stated that if the effects were related to treatment, this would be a normal and expected response.

Thyroid follicular cell hypertrophy is the normal physiological response of reduced serum T4 levels and should not be viewed as adverse. This is the typical adaptive response of a healthy normal functioning organism reacting to maintain serum T4 levels within the normal range. In contrast, an adverse effect in the thyroid would be a failure or an inability to respond to changes in T4 levels. The fact that T4 levels in the treated groups were comparable to control levels at week 17 indicates that any effect was reversible.

Amacher DE, Schomaker SJ and Burkhardt JE. 1998. The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies. Food Chem. Toxicol. 36:831-839.

Capen CC. 1997. Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicol. Pathol. 25:39-48.

Williams GM and Iatropoulos MJ. 2002. Alteration of liver cell function and proliferation: differentiation between adaptation and toxicity. Toxicol Pathol. 2002 Jan-Feb;30 (1):41-53.

Point 2: Statistically significant ($p<0.05$ or $p<0.01$) clinical chemistry changes accompanied by histopathological changes in the liver in approximately 50% of males in the 100, 300 and 1000 mg/kg/day dose groups and in the 300 and 1000 mg/kg/day female dose groups; a statistically significant ($p<0.05$ or $p<0.01$) increase in the mean liver weights at all dose levels of both males and females.

Clinical Chemistries

The Agency did not specify which clinical chemistries were of interest in relation to histopathological changes in the liver. We believe the clinical chemistries of interest are albumin, globulin, total protein, and GGT based on the Agency's comment regarding hepatic histopathology.

Please see pages 44-46 of the final report for a discussion of the changes in serum chemistries observed in this study. Pages 1656 and 1666 of the Addendum (July 2002) provide the test laboratory's historical control data for age and sex matched serum Gamma Glutamyltransferase (GGT) levels in this strain of rats.

The HBCD study evaluated the clinical chemistry parameters that are typical of 90-day rat studies. Those related to liver function included Albumin, Total Protein, Globulin, A/G Ratio, Total Bilirubin, Alkaline Phosphatase (AP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), GGT, Glucose, and Total Cholesterol. Of these, a statistical difference from the control value was detected at week 13 in some treated groups for albumin levels (increased), globulin (increased), total protein, and GGT (increased).

These statistical differences were not those characteristic of an adverse effect on the liver. Hepatotoxicity would be expected to decrease, not increase, albumin, globulin and total protein. Certain forms of hepatotoxicity may result in increased serum GGT levels. However, it would be unusual to observe an increase in serum GGT as the sole indicator of hepatotoxicity, as was the case in this study. Changes in clinical chemistries indicative of hepatotoxicity typically include increases in several hepatic enzymes (e.g. AP, ALT, and AST) as well changes in hepatic function as indicated by serum protein (decreased) and bilirubin (increased) levels. In the HBCD 90-day study, serum AP, ALT and AST levels were not affected in treated rats. Serum protein levels in treated animals were increased, not decreased. Serum bilirubin levels were comparable in treated and control animals. Further, while some statistically significant differences in serum GGT levels were detected between control and treated groups, the serum GGT levels at week 13 were within the range of the historical control values in this age and strain of rat for this laboratory (see pages 1656 and 1666 of the July 2002 Addendum).

Finally, hepatic GGT can be inducted in the rat (Teschke et al 1983; Chandar and Nagarajan 1984), and such an induction has been shown to increase serum GGT levels in the rat (Goldberg 1980; Teschke et al. 1983; Nishmura and Teschke 1982; Satoh et al. 1982).

Liver Histopathology

The Agency did not specify which histopathological changes were of interest; however, we believe the referred to change is hepatocellular vacuolation.

Please see pages 51-54 of the final report for a discussion of the microscopic examination of tissues from this study.

The histopathological change of minimal hepatocellular vacuolation (HCV) was reported at 50, 40 and 55% in males at dose-levels of 100, 300 and 1000 mg/kg/d, respectively. The incidence of minimal HCV in control males was 10%. The incidence of mild HCV in the 0, 100, 300 and 1000 mg/kg groups was 10, 10, 10, and 11%, respectively. Thus, only minimal HCV appeared to be increased in incidence in treated animals. However, there was no dose response and the incidence of minimal HCV was approximately equivalent across all dose levels. HCV of minimal severity and observed with no dose response is not indicative of toxicity, particularly when viewed in relation to the absence of adverse effects on clinical chemistries.

The histopathological change in females at dose-levels of 0, 100, 300, and 1000 mg/kg/d consisted of minimal HCV in 30, 60, 30 and 50%, respectively. Mild HCV and moderate HCV was found at an incidence of 0, 0,10 and 20%, respectively. The severity and incidence of the hepatocellular vacuolation is not indicative of toxicity, particularly when viewed in relation to the absence of adverse effects on clinical chemistries.

Liver weights

Please see page 50 of the final report for a discussion of liver weights. Mean liver weights were increased in the male and female at dose-levels of 100, 300, 1000 mg/kg/d. The liver weights returned completely, or near completely, to that of the control group by week 17.

In the rat, an increase in liver weight frequently accompanies enzyme induction. Recent work on the relationship of liver weight, microsomal enzyme induction, and histological change in rat toxicology studies concludes "The preponderance of data collected in these 11 studies indicates that microsomal enzyme induction was not accompanied by evidence of chemically-induced liver injury. We conclude that in the rat, both hepatomegaly and microsomal enzyme induction are benign and adaptive changes in response to certain chemicals that stimulate the hepatic drug metabolizing enzyme system." (Amacher et al. 1998).

Limited pharmacokinetic studies indicate HBCD is extensively metabolized prior to excretion in the feces and urine. The results of the fat analysis in this study indicate that mammalian system handles the 3 HBCD stereoisomers differently, and may be less efficient at eliminating one stereoisomer over another. (The relative isomer concentrations in adipose tissue at all time points were alpha>>gamma>beta in contrast to the test article composition of gamma>>alpha>beta.) Thus, it should not be

unexpected that hepatic enzyme induction occurs with exposure to substantial doses over a significant period of time, as was the case in the 90-day study.

In conclusion, the changes seen in this study, the increase in liver weight, the lack of adverse histologic effects in the liver and the apparent normal functioning of the liver, are consistent with enzyme induction (Amacher et al. 1998). Hepatic enzyme induction is an adaptive, and not an adverse, effect (Williams and Iatropoulos 2002).

Amacher DE, Schomaker SJ and Burkhardt JE. 1998. The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies. Food Chem. Toxicol. 36:831-839.
Chandar N and Nagarajan B. 1984. Induction of gamma-glutamyl transpeptidase by hexachlorocyclohexane. Biochem Int. Jan;8 (1):41-8.

Goldberg DM. 1980. Structural, functional, and clinical aspects of gamma-glutamyltransferase. CRC Crit Rev Clin Lab Sci. 12 (1):1-58.

Nishmura M and Teschke R. 1982. Effect of chronic alcohol consumption on the activities of liver plasma membrane enzymes: gamma-glutamyltransferase, alkaline phosphatase and 5'-nucleotidase. Biochem Pharmacol. Feb 1; 31(3):377-81.

Satoh T, Igarashi T, Hirota T and Kitagawa H. 1982. Induction of hepatic gamma-glutamyl transpeptidase in rats by repeated administration of aminopyrine. J Pharmacol Exp Ther. Jun; 221(3):795-800.

Teschke R, Neuefeind M, Nishimura M and Strohmeryer G. 1983. Hepatic gamma-glutamyltransferase activity in alcoholic fatty liver: comparison with other liver enzymes in man and rats. Gut. 24 (7):625-30.

Williams GM and Iatropoulos MJ. 2002. Alteration of liver cell function and proliferation: differentiation between adaptation and toxicity. Toxicol Pathol. 2002 Jan-Feb; 30 (1):41-53.

Point 3: Increase in mean prostate weight in the 1000 mg/kg/day male dose group.

The mean prostate weight in males of the 1000 mg/kg/d dose group was statistically greater than that of the control mean at week 13. At week 17, after a 28-day recovery period, the mean in this dose group was statistically comparable to the control mean. The effect was reversible. No histopathologic evidence of an adverse effect on the prostate in this or any other dose group, and extensive analysis of seminal quality showed no difference in control and treated groups. Thus, no adverse effect of treatment was found on the prostate.

Point 4: The 28-day post-exposure recovery period showed that some of the above effects were not reversible.

The Agency did not specify what effects were not reversible. We believe the Agency is likely referring to liver, thyroid/parathyroid and prostate weights and histopathology. At the recovery sacrifice at week 17, serum T4 and clinical chemistry levels in treated animals were comparable to control values.

Organ Weights

The organs of interest are the liver, prostate and thyroid/parathyroids based on week 13 data. At week 17, e.g. after the 28 day recovery period, the weights of these organs were comparable in control and treated groups with the exception the thyroid/parathyroid weights in females in the 300 and 1000 mg/kg/d groups (Tables 1, 2). At week 17, the mean thyroid/parathyroid weights in these two groups were greater than those of the control mean (Table 2). However, these week 17 weights were less than the corresponding week 13 means in all 3 treated groups, e.g. the thyroid/parathyroid glands were reduced in size from that seen after 13 weeks of treatment. This is indicative of recovery.

The detection of a statistical difference in the mean organ weight in the 300 and 1000 mg/kg/day groups vs. the control group is likely due to an atypically low mean in the control group. The mean thyroid/parathyroid weight in the control group at week 17 was 0.016 grams vs. 0.023 grams at week 13. Thyroid weight in the control group at week 17 is expected to be roughly equivalent to that at week 13; however, in this case thyroid weight at week 17 was less than that at week 13.

Thus, the three organs differing from the control mean at week 13, were the liver, prostate and thyroid/parathyroids. The mean liver and prostate weights in the treated animals was comparable to the control mean at week 17, e.g. after a 28-day recovery period. The same is true for thyroid/parathyroid weight in the males. In the females, a statistically significant difference was detected between the mean of the 300 and 1000 mg/kg dose groups and the controls. However, in all 3 female dose groups, the week 17 thyroid/parathyroid weights decreased from their respective week 13 means. That the decline in thyroid weight after dosing stopped is indicative of recovery, and the statistical significance at the mid and high doses is likely due to the atypically low control mean (0.016 g) rather than a true biological effect.

Therefore, we conclude that the effect on liver, prostate and thyroid/parathyroid weight was reversible.

Table 1. Mean Absolute Organ Weights in Male Rats.

	Liver		Prostate		Thyroid	
	Wk 13	Wk 17	WK 13	Wk 17	Wk 13	Wk 17
0	14.3	14.4	0.95	1.2	0.023	0.029
100	17.0*	14.7	0.99	0.9	0.027	0.031
300	17.2*	15.7	1.12	1.1	0.026	0.028
1000	19.0**	14.1	1.25*	1.1	0.023	0.028

Table 2. Female Rats, Mean Absolute Organ Weights.

	Liver		Prostate		Thyroid	
	Wk 13	Wk 17	WK 13	Wk 17	Wk 13	Wk 17
0	8.12	8.1	-	-	0.023	0.016
100	9.92*	7.6	-	-	0.028	0.020
300	10.6*	8.8	-	-	0.026	0.022*
1000	12.4**	9.1	-	-	0.023	0.022*

Histopathology

Please see page 51-54 of the final report.

No histologic changes were observed in the prostate of treated groups at week 13 or 17.

At week 17, the changes observed in livers of some treated groups had resolved without any delayed or long-term toxic effects.

At week 17, the mild changes in the thyroid of some animals observed at 13 weeks had resolved in all treated males and in the 300 mg/kg/d females. These changes had nearly completely resolved in the 1000 mg/kg/d females.

Thus, the only histologic effect observed at week 13, but not completely resolved at week 17, was mild thyroid follicular hypertrophy in the high dose female group. This effect had diminished in these animals, and had resolved in the next lower dose group. The lack of complete resolution of this effect was a function of time and dose, and not of irreversibility.

Point 5. The results of the body fat HBCD residue satellite study (only performed with the high dose group) shows substantial HBCD levels throughout the 90-day study as well as at the end of the 28-day exposure period.

Detection of a chemical in a tissue/organ is not, in and of itself, an adverse effect. Thus, detectable levels of the test article in tissues at the end of a 90-day study are not used to determine a no-adverse-effect-level. Although detectable levels remained after a 28-day recovery period, the levels had clearly declined during that period. Thus, the test animals were able to eliminate the test article from the body once exposure to the very high dose level, 1000 mg/kg/d, had ceased. No adverse effects were seen in the recovery group animals.

ATTACHMENT II

Although the HBCD 90-Day Study in Rats was submitted to the Agency voluntarily by ACC, the study was not required to be submitted to EPA pursuant to TSCA § 8(e) because the data do not reasonably support the conclusion that the substance presents a substantial risk of injury to health or the environment. Moreover, EPA already had in its possession studies which are consistent with the data and the conclusions reached in the study. The following provides a more detailed discussion of the comparability of the data and conclusions reached in the HBCD 90-Day Study in question with information contained in studies previously reviewed by the Agency.

HBCD has been tested in 4 subchronic toxicity studies: a 1969 28-day study in rats, a 1970 90-day study in rats, a 1996 28-Day study in rats, and a 2001 90-day study in rats, which is under discussion here. With the exception of the 2001 90-day study in rats, these studies are included in the TSCATS database, and the dates of receipt were 1990, 1997 and 1998. Please note that the TSCATS database contains at least 2 files for HBCD: one under the Chemical Name Hexabromocyclododecane, and another under the CAS Number 003194556.

The results in all 4 subchronic studies, particularly with respect to organ weight and histopathology, are remarkably consistent when compared by dose level and duration of dosing. (The results of 1996 and 1969 28-day studies and the 1970 90-day study are summarized below.) These results in the 2001 Rat 90-day study do not represent new information for HBCD or the Agency.

Serum thyroid hormone levels were measured only in the 2001 90-day study. The statistical decrease in serum T4 levels, at week 13, but not at week 3 or 17, were not accompanied by systemic indications of thyroid disease or thyroid insufficiency, were reversible, and thus were not considered to represent an adverse effect. Treated animals were of normal weight, had normal weight gain, normal motor activity and functional observational battery results, normal hematology and urinalysis, and normal ocular exams, estrus cycles, and sperm analysis. The decrease in serum T4 levels was associated with hepatic enzyme induction and enhanced elimination of T4. While the rat is particularly sensitive to this effect, humans are not. Further, it is improbable that exposure to doses as high as 100 mg/kg/d for 90 consecutive days (minimum dose and duration resulting in a statistical decrease in serum T4 levels in the rat) could occur anywhere outside of the test laboratory.

SUMMARIES OF EXISTING SUBCHRONIC STUDIES ON HBCD IN THE RAT

1. 1996 Rat 28 Day.

TSCATS Database Document #s FYI-OTS-0397-1289, Received 3/31/1997; 86-980000155, Received 6/22/1998; 86970000747, Received 3/25/1997; and FYI-OTS-0898-0898-1289, Received 8/4/1998. The multiple documents appear to be duplications of 2 original documents.

HBCD, in the vehicle corn oil, was administered orally by gavage to three groups of Sprague-Dawley Crl: CD BR rats for a period of 28 consecutive days. Dose levels were 125 (low), 350 (mid), or 1,000 (high) mg/kg/day, administered at dosage volume of 5 ml/kg. The test groups consisted of 6 males and 6 females in the 125 and 350 mg/kg/day groups and 12 males and 12 females in the 1000 mg/kg/day group. A concurrent control group comprised of 12 males and 12 females received the vehicle, corn oil, for 28 consecutive days at a dosage volume of 5 ml/kg. At the end of the dosing period, 6 animals/sex/group were sacrificed and necropsied. The remaining 6 animals/sex in the control and 1000 mg/kg/day groups remained on-test untreated for a 14-day recovery period. At the end of the recovery period, all animals were sacrificed and necropsied.

Animals were observed twice daily for mortality and moribundity. Clinical signs were recorded daily. Body weight and food consumption were measured weekly. Functional observational battery and motor activity evaluations were performed during weeks -1 (pretest), 3, and 5 (recovery). Samples for hematology and serum chemistry evaluations were collected at the primary (28 day) and recovery (42 day) sacrifices. Complete necropsies were performed on all rats. The brain, liver, kidney, heart, spleen, testes and epididymus or ovaries, adrenal glands, and thymus from all animals were weighed at each sacrifice. Approximately 40 tissues were collected and preserved at each necropsy from all animals. The following tissues were examined microscopically from the control and high dose animals: liver, kidney, heart, spleen, testes (males), prostate (males), seminal vesicles (males), epididymus (males), ovaries (females), adrenal glands, thymus, bone with marrow (sternebra), brain, stomach, cecum, duodenum, ileum, jejunum, lymph node, peripheral nerve (sciatic), spinal cord, lung, trachea, uterus (females), urinary bladder, and all gross lesions. The lungs, liver, kidneys, stomach, thyroid, gross lesions and target organs were examined in all dose levels.

Survival was not affected by administration of the test article. All animals survived to the scheduled sacrifice. Clinical signs observed during the study were nonspecific, low in incidence, non-dose-related and not considered related to test article.

Body weights, weight gain and food consumption of treated animals were compared statistically by sex and treatment day to their respective control groups ($p \leq 0.05$ or 0.01) and were not affected by treatment. No statistically significant differences in body weight between control and treated animals were detected with the exception of an increase in mean female body weight in the 350 mg/kg/day group during week 2 of treatment. Mean female body weight at that time point was 196 g versus 179 g in the

control group. No statistically significant differences in body weight gain between control and treated animals were detected with the exception of a decrease in mean male body weight gain in the 1,000 mg/kg/day recovery group during week 1 of recovery. Mean male body weight gain at that time point was 21 g versus 31 g in the control group; mean male body weight was not statistically different from the control mean. No statistically significant differences in food consumption between control and treated animals were detected with the exception of an increase in mean female food consumption in the 350 mg/kg/day group during weeks -1, 1, and 2 of treatment. Mean female food consumption at that those time points were 18, 17 and 17 g versus16, 15 and 15 g in the control group, respectively.

Functional observation battery and motor activity results from treated animals were compared statistically by sex and treatment day to their respective control groups ($p \leq 0.05$). These parameters were not affected by treatment with the test article. No statistically significant differences were observed between treated and control animals at any time point.

No statistically significant differences between treated and control animals were found for hematology parameters with the exception of an increase in the mean activated partial thromboplastin time in the 1000 mg/kg/day males on week 4 and a decrease in the mean prothrombin time in the 1000 mg/kg/day females on week 4. These statistical differences were not of toxicological significance.

No toxicologically significant effects on serum chemistry values related to test article administration were observed at the 28-day primary and 42-day recovery sacrifice. Scattered instances of statistically significant differences between treated and control animals were detected for some serum chemistry parameters at the 28-day primary sacrifice. These scattered statistical differences were not considered toxicologically significant because the statistical differences occurred: in the absence of a dose response, in the absence of the accompanying clinical chemistry changes expected, in the opposite direction from what occurs in a toxic state, in a direction which is without physiologic significance, or due to potential interference with the laboratory method. No statistically significant differences in serum chemistry parameters were detected between groups at the 42-day recovery sacrifice.

No gross lesions that could be attributed to the test article were detected at either necropsy. Gross lesions were nonspecific, low in incidence, non-dose-related and considered incidental.

No microscopic lesions that could be attributed to the test article were detected on histopathologic exam. Microscopic changes were nonspecific, low in incidence, non-dose-related and considered incidental.

No statistical significant differences in organ weight or organ to body weight ratios were detected between control and treated animals with one exception. Absolute liver weights were statistically significantly increased with respect to control means at the 28-day

sacrifice in males in the high dose and females in the mid and high dose. Liver to body weight ratios in mid and high dose males and low, mid and high dose females were statistically significantly increased at the 28-day sacrifice. At the recovery sacrifice, male absolute and liver to body weight ratio were statistically comparable to the control mean whereas female absolute liver weights and liver to body weight ratio were statistically significantly increased with respect to control mean. The difference in absolute liver weight between control and treated females was less pronounced at the end of the recovery period, indicating the increase in liver weight was reversible in females as well as males. In the absence of test article related histopathologic and serum chemistry changes, increases in liver weight are considered an adaptive, rather than a toxic response, are not uncommon in the rat, and are most likely the result of microsomal induction.

In conclusion, no systemic toxicity was observed at any dose level. Based on the results of this study, the NOAEL (No Observed Adverse Effect Level) of HBCD administered orally to male and female rats for 28 consecutive days was 1,000 mg/kg/day *(Chengelis C. 1996 A 28-day repeated dose oral toxicity study of HBCD in rats. Study No. WIL-186004. WIL Research Laboratories, Inc. Ashland, OH).*

2. 1969 Rat 28-day Study

TSCATS Database Document # 86-900000376; Received 4/5/1990.

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats (10/sex/group) at doses of 0, 1, 2.5 and 5% of the diet for 28 days. Doses calculated from the actual body weights and food consumption in this study are 0, 940, 2410, and 4820 mg/kg body weight/day.

No clinical signs related to treatment were observed at the 1% dose level. Body weights at the 1 and 2.5% dose levels were comparable to the controls. Liver weights (absolute and relative to body weight) were increased at all dose levels, but no microscopic pathology was detected. Thyroid hyperplasia was observed in some animals at all doses, and "very slight numerical development of the follicles and ripening follicles in the ovaries of females" at the high dose (4820 mg/kg/d) was reported. No changes in any other organ related to treatment and no changes in clinical chemistry tests were detected.

The report concluded that "The increased liver weight must be attributed to hyperactivity; hypermetabolism as a result of increased thyroid activity appears probable in view of the observations of the thyroid". Therefore, the increased liver weights were not pathologic: there were no microscopic lesions detected on histopathology and no change in clinical chemistry values *(Zeller H and Kirsch P (1969) Hexabromocyclododecane: 28-day feeding trials with rats. BASF Unpublished Laboratory Report).*

3. 1970 Rat 90-day Study

TSCATS Database Document # 86-900000380; Received 4/5/1990.

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats at doses of 0, 0.16, 0.32, 0.64 and 1.28% of the diet for 90 days. Doses calculated on the actual body weights and food consumption in this study reveals: 0, 120, 240, 470 and 950 mg/kg body weight/day.

Doses up to 0.64% (470 mg/kg/d) produced no adverse clinical signs, no change in body weight, and no change in clinical chemistry results. An increase in the relative liver to body weight ratio was found, and was accompanied by fatty accumulation but no other histologically discernible changes were detected in the liver. Further, no histological changes were found in any other organ. The original report stated that in the "absence of detectable clinico-chemical disturbances or histological changes of the vital organs, it was concluded that the increased liver weight and the fat deposits, both of which were largely reversible when administration of Hexabromid S was stopped, were the result of a temporary increase in the activity of the liver." Thus, no adverse effect was produced at the highest dose tested, 1.28% of the diet *(Zeller H and Kirsch P (1970) Hexabromocyclododecane: 90-day feeding trials with rats. BASF Unpublished Laboratory Report).*